In the Sequence Listing:

Please insert the attached paper copy of the Sequence Listing as new pages 1-27 in the above-captioned application. A request to use the computer-readable copy (CFR copy) of the Sequence Listing from the parent application accompanies this response.

see B1

Amendments

In the Specification:

Please replace the paragraph beginning at page 12, line 24, with the following rewritten paragraph:

-- The sequences of these genes are shown in Example 1 in one contiguous sequence or contig of 71,989 nucleotides (SEQ ID NO:2). This contig also contains two genes that appear to originate from a transposon and are identified below as ORF A and ORF B. These two genes are believed not to be involved in epothilone biosynthesis but could possibly contain sequences that function as a promoter or enhancer. The contig also contains more than 12 additional ORFs, only 12 of which, designated ORF2 through ORF12 and ORF2 complement, are identified below. As noted, ORF2 actually is two ORFs, because the complement of the strand shown also comprises an ORF. The function of the corresponding gene product, if any, of these ORFs has not yet been established. The Table below provides the location of various open reading frames, module-coding sequences, and domain encoding sequences within the contig sequence shown in Example 1. Those of skill in the art will recognize, upon consideration of the sequence shown in Example 1, that the actual start locations of several of the genes could differ from the start locations shown in the table, because of the presence in frame codons for methionine or valine in close proximity to the codon indicated as the start codon. The actual start codon can be confirmed by amino acid sequencing of the proteins expressed from the genes. --

B2

Please replace the paragraph beginning at page 18, line 16, with the following rewritten paragraph:

-- The loading domain of the epothilone PKS includes an inactive KS domain, KS^Y, an AT domain specific for malonyl CoA (which is believed to be decarboxylated by the KS^Y domain to yield an acetyl group), and an ACP domain. The present invention provides recombinant DNA compounds that encode the epothilone loading domain. The loading domain coding sequence is contained within an ~8.3 kb EcoRI restriction fragment of cosmid pKOS35-70.8A3. The KS domain is referred to as inactive, because the active site region "TAYSSSL" (SEQ ID NO:20) of the KS domain of the loading domain has a Y residue in place of the cysteine required for ketosynthase activity; this domain does have decarboxylase activity. See Witkowski *et al.*, 7 Sep. 1999, Biochem. 38(36): 11643-11650, incorporated herein by reference. --

Please replace the paragraph beginning at page 19, line 11, with the following rewritten paragraph:

-- The epothilone loading domain also contains an AT domain believed to bind malonyl CoA. The sequence "QTAFTQPALFTFEYALAALW. . . GHSIG" (SEQ ID NO:1) in the AT domain is consistent with malonyl CoA specificity. As noted above, the malonyl CoA is believed to be decarboxylated by the KS^Y domain to yield acetyl CoA. The present invention provides recombinant epothilone derivative loading domains or their encoding DNA sequences in which the malonyl specific AT domain or its encoding sequence has been changed to another specificity, such as methylmalonyl CoA, ethylmalonyl CoA, and 2-hydroxymalonyl CoA. When expressed with the other proteins of the epothilone PKS, such loading domains lead to the production of epothilones in which the methyl substituent of the thiazole ring of epothilone is replaced with, respectively, ethyl, propyl, and hydroxymethyl. The present invention provides recombinant PKS enzymes comprising such loading domains and host cells for producing such enzymes and the polyketides produced thereby. --

Please replace the paragraph beginning at page 73, line 7, with the following rewritten paragraph:

-- To screen the library, two segments of KS domains were used to design oligonucleotide primers for a PCR with *Sorangium cellulosum* genomic DNA as template. The fragment generated was then used as a probe to screen the library. This approach was chosen, because it was found, from the examination of over a dozen PKS genes, that KS domains are the most

highly conserved (at the amino acid level) of all the PKS domains examined. Therefore, it was expected that the probes produced would detect not only the epothilone PKS genes but also other PKS gene clusters represented in the library. The two degenerate oligonucleotides synthesized using conserved regions within the ketosynthase (KS) domains compiled from the DEBS and soraphen PKS gene sequences were (standard nomenclature for degenerate positions is used): CTSGTSKCSSTBCACCTSGCSTGC (SEQ ID NO:21) and TGAYRTGSGCGTTSGTSCCGSWGA (SEQ ID NO:22). A single band of ~750 bp, corresponding to the predicted size, was seen in an agarose gel after PCR employing the oligos as primers and *S. cellulosum* SMP44 genomic DNA as template. The fragment was removed from the gel and cloned in the HincII site of pUC118 (which is a derivative of pUC18 with an insert sequence for making single stranded DNA). After transformation of *E. coli*, plasmid DNA from ten independent clones was isolated and sequenced. The analysis revealed nine unique sequences that each corresponded to a common segment of KS domains in PKS genes. Of the nine, three were identical to a polyketide synthase gene cluster previously isolated from this organism and determined not to belong to the epothilone gene cluster from the analysis of the modules. The remaining six KS fragments were excised from the vector, pooled, end-labeled with ^{32}P and used as probe in hybridizations with the colonies containing the cosmid library under high stringency conditions. --

B5 con't

Please replace the paragraph beginning at page 79, line 14, with the following rewritten paragraph:

-- The sequence of the epothilone PKS and flanking regions has been compiled into a single contig, as shown below (SEQ ID NO:2). --

B6

Please replace the paragraph beginning at page 102, line 8, with the following rewritten paragraph:

-- The present invention also provides expression vectors in which the recombinant PKS genes of the invention are under the control of a *Myxococcus xanthus* promoter. To construct an illustrative vector, the promoter of the pilA gene of *M. xanthus* was isolated as a PCR amplification product. Plasmid pSWU357, which comprises the pilA gene promoter and is

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described in Wu and Kaiser, Dec. 1997, J. Bact. 179(24):7748-7758, was mixed with PCR primers Seq1 and Mxpil1 primers:

Seq1: 5'-AGCGGATAACAATTTCACACAGGAAACAGC-3' (SEQ ID NO:3); and

Mxpil1: 5'-TTAATTAAGAGAAGGTTGCAACGGGGGGC-3' (SEQ ID NO:4),

and amplified using standard PCR conditions to yield an ~800 bp fragment. This fragment was cleaved with restriction enzyme KpnI and ligated to the large KpnI-EcoRV restriction fragment of commercially available plasmid pLitmus 28 (New England Biolabs). The resulting circular DNA was designated plasmid pKOS35-71B. --

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Please replace the paragraph beginning at page 103, line 4, with the following rewritten paragraph:

-- The sequence of the pilA promoter in these plasmids is shown below (SEQ ID NO:5). --

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Please replace the paragraph beginning at page 113, line 17, with the following rewritten paragraph:

-- To improve production of epothilones from these vectors, the eryKS5 linker sequences were replaced by epothilone PKS gene coding sequences, and the vectors were introduced into *Streptomyces coelicolor* CH999. To amplify by PCR coding sequences from the *epoA* gene coding sequence, two oligonucleotides primers were used:
N39-73, 5'-GCTTAATTAAGGAGGACACATATGCCCGTCGTGGCGGATCGTCC-3' (SEQ ID NO:6); and N39-74, 5'-GCGGATCCTCGAATCACCGCCAATATC-3' (SEQ ID NO:7).
The template DNA was derived from cosmid pKOS35-70.8A3. The ~0.8 kb PCR product was digested with restriction enzymes PacI and BamHI and then ligated with the ~2.4 kb BamHI-NotI and the ~6.4 kb PacI-NotI restriction fragments of plasmid pKOS039-120 to construct plasmid pKOS039-136. To make the expression vector for the *epoA*, *epoB*, *epoC*, and *epoD* genes, the ~5 kb PacI-AvrII restriction fragment of plasmid pKOS039-136 was ligated with the ~50 kb PacI-AvrII restriction fragment of plasmid pKOS039-124 to construct the expression plasmid pKOS039-124R. Plasmid pKOS039-124R has been deposited with the ATCC under the terms of the Budapest Treaty and is available under accession number _____. --

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Please replace the paragraph beginning at page 114, line 3, with the following rewritten paragraph:

B10

-- To amplify by PCR sequences from the *epoE* gene coding sequence, two oligonucleotide primers were used: N39-67A, 5'-GCTTAATTAAGGAGGACACATATGACCGACCGAGAAGGCCAGCTC-CTGGA-3' (SEQ ID NO:8), and N39-68, 5'-GGACCTAGGCGGGATGCCGGCGTCT-3' (SEQ ID NO:9). --

Please replace the paragraph beginning at page 114, line 21, with the following rewritten paragraph:

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-- The *epoK* gene sequences were amplified by PCR using the oligonucleotide primers: N39-69, 5'-AGGCATGCATATGACCCAGGAGCAAGCGAATCAGAGTG-3' (SEQ ID NO:10); and N39-70, 5'-CCAAGCTTTATCCAGCTTTGGAGGGCTTCAAG-3'(SEQ ID NO:11). --

Please replace the paragraph beginning at page 114, line 25, with the following rewritten paragraph:

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-- The *epoL* gene sequences were amplified by PCR using the oligonucleotide primers: N39-71A, 5'-GTAAGCTTAGGAGGACACATATGATGCAACTCGCGCGCGGGTG-3' SEQ.ID.NO.12) ; and N39-72, 5'-GCCTGCAGGCTCAGGCTTGCGCAGAGCGT-3' (SEQ ID NO:13). --

Please replace the paragraph beginning at page 118, line 23, with the following rewritten paragraph:

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-- Another method to increase the yield of epothilones produced is to change the KS^Y domain of the loading domain of the epothilone PKS to a KS^Q domain. Such altered loading domains can be constructed in any of a variety of ways, but one illustrative method follows. Plasmid pKOS39-124R of the invention can be conveniently used as a starting material. To amplify DNA fragments useful in the construction, four oligonucleotide primers are employed: N39-83: 5'-CCGGTATCCACCGCGACACACGGC-3' (SEQ ID NO:14), N39-84: 5'-GCCAGTCGTCCTCGCTCGTGGCCGTTC-3' (SEQ ID NO:15),

and N39-73 and N39-74, which have been described above. The PCR fragment generated with N37-73 and N39-83 and the PCR fragment generated with N39-74 and N39-84 are treated with restriction enzymes PacI and BamHI, respectively, and ligated with the ~3.1 kb PacI-BamHI fragment of plasmid pKOS39-120 to construct plasmid pKOS039-148. The ~0.8 kb PacI-BamHI restriction fragment of plasmid pKOS039-148 (comprising the two PCR amplification products) is ligated with the ~2.4 kb BamHI-NotI restriction fragment and the ~6.4 kb PacI-NotI restriction fragment of plasmid pKOS39-120 to construct pKOS39-136Q. The ~5 kb PacI-AvrII restriction fragment of plasmid pKOS039-136Q is ligated to the ~50 kb PacI-AvrII restriction fragment of plasmid pKOS039-124 to construct plasmid pKOS39-124Q. Plasmids pKOS039-124Q and pKOS039-126R are then transformed into *Streptomyces coelicolor* CH999 for epothilone production. --

Please replace the paragraph beginning at page 120 line 26, with the following rewritten paragraph:

-- Plasmids were constructed to encode fusion proteins composed of six histidine residues fused to either the amino or carboxy terminus of EpoK. The following oligos were used to construct the plasmids:

55-101.a-1:

5'-AAAAACATATGCACCACCACCACCACCACATGACACAGGAGCAAGCGAAT-CAGAGTGAG-3' (SEQ ID NO:16),

55-101.b:

5'-AAAAAGGATCCTTAATCCAGCTTTGGAGGGCTT-3' (SEQ ID NO:17),

55-101.c:

5'-AAAAACATATGACACAGGAGCAAGCGAAT-3' (SEQ ID NO:18), and

55-101.d:

5'-AAAAAGGATCCTTAGTGGTGGTGGTGGTGGTGTCCAGCTTTGGAGGGCTTC-AAGATGAC-3' (SEQ ID NO:19). --